UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Review of Additional Insect Resistance Management (IRM) information for SmartStax (MON 89034 x TC1507 x MON 88017 x DAS 59122-7) Bt corn. EPA Reg No. 524-LIR. No MRID#. Decision#: 394799. DP Barcode: 366443.

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Action Requested

BPPD[1] has been asked to review additional information on insect resistance management (IRM) submitted by Monsanto Company to support registration of SuperStax Bt corn (EPA Reg. No. 524-LIR). This information was submitted in response to a deficiency letter issued by EPA on March 19, 2009. SmartStax is a joint registration effort between Monsanto and Dow AgroSciences (Monsanto is the data submitter) and contains the following previously-registered events: MON 89034 (Monsanto), TC1507 (Dow), MON 88017 (Monsanto), and DAS 59122-7 (Dow). The new IRM information is contained in a document titled "Response to U.S. EPA BPPD letter, dated March 19, 2009 regarding applications to register MON 89034 x TC1507 x MON 88017 x DAS 59122-7..." (dated April 9, 2009). No MRID number was assigned to this submission. Additional information from Monsanto/Dow was submitted via e-mail (Russ Schneider to Alan Reynolds, dated 6/15/09).

[1] The use of BPPD in this review refers to the BPPD IRM Team consisting of Alan Reynolds and Jeannette Martinez

Conclusions and Recommendations

IRM and Refuge Recommendations for SmartStax Corn

1) Overall, Monsanto/Dow have provided sufficient scientific justification to support a reduced corn rootworm (CRW) refuge of 5% for SmartStax Bt corn. Data reviewed by BPPD here and previously (see BPPD 2009) include studies on dose for CRW, cross resistance between the Cry3Bb1 and Cry34/34 toxins, and simulation modeling to predict potential resistance evolution. However, BPPD notes that there is still uncertainty regarding the true CRW dose expressed by SmartStax (and its single trait components) and worst case simulation modeling (as described in the conclusions below). Therefore, BPPD recommends that additional data be generated (as described below) to verify and further buttress the current data supporting lower refuge. Such data could be submitted as a term or condition of approval or by other means after registration. BPPD also strongly recommends a post-registration assessment of the lower refuge within several years of approval once any new data have been submitted and reviewed.

2) Monsanto/Dow have previously provided data to support a 5% refuge for lepidopteran target pests of SmartStax corn (see BPPD 2009). If a SmartStax registration is granted with a 5% CRW refuge, growers will be able to plant a combined refuge of 5% for both target pests. The deployment requirements for the current (20%) CRW refuge are still applicable for a 5% refuge including distance (adjacent to or within Bt fields) and row width for strip refuges (at least 4 rows wide).

3) Given that SmartStax will likely have different refuge strategies for lepidoptera and CRW than other registered Bt corn products, BPPD recommends that Monsanto/Dow submit a revised compliance plan. This strategy should be specific for SmartStax and the new refuge requirements. Compliance is an area of ongoing concern -- recent data have shown that refuge compliance for Bt corn has fallen in recent years.

4) Existing programs for resistance monitoring and remedial action that were established for MON 89034 (Cry1A.105 and Cry2Ab2), MON 88017 (Cry3Bb1), and Herculex Xtra (Cry1F and Cry34/35) should be applicable to SmartStax corn. In light of potentially lower overall structured Bt corn structured refuge, BPPD recommends that the CRW resistance monitoring program be expanded (i.e. with additional sampling and collection sites or improved monitoring techniques). Also, a revised definition of "resistance" may be needed for the CRW monitoring and remedial action plans based on recent research and selection experiments (Lefko et al. 2008; Meihls et al. 2008).

Conclusions Regarding Dose, Resistance Allele Frequency, and Modeling Data

5) BPPD agrees with Monsanto/Dow that the methodology used to calculate dose for SmartStax (developed in Storer et al. 2006 and used in Hucakaba and Storer 2008) is a reasonable approach to addressing dose for CRW. There is some conflicting evidence about the effect of density dependent mortality on dose calculations; BPPD agrees with Monsanto/Dow's use of the data from the Huckaba and Storer (2008) study that was not

adjusted for density dependent effects. These more conservative dose estimates (96.17 - 99.96% for Cry3Bb1, 94.20 - 99.18% for Cry34/35, and 98.22 - 99.97% for Cry3Bb1 + Cry34/35 pyramid) were used in a revised model simulation.

6) Although Monsanto/Dow have used the best available dose estimates for CRW, BPPD believes that there is still uncertainty on dose in both the methodology and interpretation of available studies. This is largely due to the biology of CRW -- assessing larval response and behavior in a subterranean environment is difficult and confounding factors such as density-dependent (or independent) mortality must be considered. Storer et al. (2006) is probably the best current approach to evaluating dose, but BPPD notes that limited data have been developed using this technique (e.g. only one year with six locations of data were developed for Cry3Bb1). Other *Diabrotica* spp. may also need to be investigated: data previously submitted for northern corn rootworm revealed mortality as low as 92.8% on Cry34/35 (BPPD 2005b).

7) To address the uncertainty regarding CRW dose and buttress the dose assumptions used in the models, BPPD recommends that Monsanto/Dow provide additional dose data (using the methods of Storer et al. 2006) with Cry3Bb1 and Cry34/35. Further dose studies could also be conducted with varying egg infestation levels (above and below egg levels expected to trigger density-dependent mortality) to tease out any egg density effects. New techniques to assess CRW dose may need to be pursued as well, if Monsanto/Dow or academic researchers can develop such approaches.

8) Monsanto/Dow conducted modeling simulations to investigate the effect of initial resistance allele frequency (RAF). The results from these simulations with a pyramid showed that the initial RAF was insensitive in the model -- the final RAF did not increase significantly from the initial frequency after 10 generations of selection (regardless of the starting value). Nevertheless, BPPD is still concerned that resistance alleles for CRW-targeted Bt traits may be relatively common in the field based on published CRW selection studies (Lefko et al. 2008; Meihls et al. 2008). Monsanto/Dow's modeling has assumed an initial RAF of 0.001. This may be suitable for other pests (e.g. lepidoptera), but BPPD must consider the possibility that actual RAF for CRW is higher (perhaps close to 0.01). To further investigate this issue, BPPD recommends resistance selection experiments to further characterize putative resistance alleles and frequency of occurrence in CRW populations.

9) As with Monsanto/Dow's previous modeling, revised simulation modeling conducted with the lower dose estimates (described in #5 above) showed that resistance did not evolve to a pyramid with a 5% refuge, while single trait PIPs with a 20% refuge developed resistance in < 10 generations. An initial resistance allele frequency of 0.001 was used in the model; as discussed in #8 above, BPPD is concerned that resistance alleles may be more common among CRW in natural populations. However, BPPD notes that all modeling reviewed to date, including models submitted by Monsanto/Dow and published by independent researchers (Roush 1998; Zhao et al. 2003; Gould et al. 2006; Onstad 2009 - draft), strongly suggest that pyramided PIPs are superior to the

current single trait CRW products and can justify a lower refuge for the SmartStax pyramid.

10) BPPD recommends that new model simulations be conducted to incorporate new data (i.e. from studies conducted under #7 and 8 above) or using possible "worst case" parameters. Although Monsanto/Dow's new model simulations have been more conservative than previous runs, BPPD remains concerned that "worst case" scenarios for SmartStax have not yet been fully investigated. CRW-protected corn is highly adopted in some areas with heavy infestations so that intense selection pressure for resistance can be expected. In light of this, and the large proposed reduction in refuge (from 20% to 5%; a 75% total reduction), BPPD believes that worst case analyses are warranted to help determine the potential for resistance. In particular, model parameters for dose and initial resistance allele frequency could be adjusted to include more conservative estimates (e.g. dose ranges < 94% and RAF > 0.001).

Background

Monsanto Company and Dow AgroSciences have collaborated to develop a new Bt corn product with the trade name SmartStax. This product was developed through conventional breeding and is a combination of four previously registered Bt corn events. The components of SmartStax are summarized in table 1 below.

Table 1. Summary of the registered components of SmartStax Bt corn.

Event	Toxin(s)	Company	First Registered	Target Pests[1]	Refuge Requirements
MON 88017	Cry3Bb1	Monsanto	2005	CRW	20%
DAS 59122-7	Cry34Ab1 Cry35Ab1	Dow	2005	CRW	20%
MON 89034	Cry1A.105 Cry2Ab2	Monsanto	2008	ECB, CEW, SWCB, FAW	5% (amended from original 20%)
TC1507	Cry1F	Dow	2001	ECB, CEW, SWCB, FAW	20%

[1] Pest legend: CRW = corn rootworm (*Diabrotica* spp.); ECB = European corn borer (*Ostrinia nubilalis*); CEW = corn earworm (*Helicoverpa zea*); SWCB = southwestern corn borer (*Diatraea grandiosella*); FAW = fall armyworm (*Spodoptera frugiperda*).

Since their original registration, the four single gene events in Table 1 have been pyramided to form multi-trait products targeting both lepidoptera and corn rootworm. Monsanto developed MON 89034 x MON 88017 (trade name YieldGard Triple), which was registered in 2008 with MON 89034. Dow previously registered TC1507 x DAS 59122-7 (trade name Herculex Xtra) in 2005. SmartStax functionally combines all of these traits into one product for control of lepidoptera (European corn borer, corn earworm, southwestern corn borer, and fall armyworm) and corn rootworm.

As part of the registration application for SmartStax, an Insect Resistance Management (IRM) plan was submitted. The registrants proposed a 5% structured (non-Bt corn) refuge for both lepidopteran pests and corn rootworm (CRW). BPPD reviewed the proposal (BPPD 2009) and concluded that: 1) a 5% refuge for lepidoptera could be supported (largely based on an analysis conducted for MON 89034 x MON 88017), and 2) a 5% refuge for CRW could not be supported due to uncertainties with the submitted data. Specifically, BPPD identified the following concerns for a 5% CRW refuge:

- Dose assumptions for the SmartStax toxins (Cry3Bb1 and Cry34/35) that were used in simulation models for SmartStax may have been unrealistic. These models assumed high mortalities (mostly >99%) for the individual Cry3Bb1 and Cry34/35 toxins, based largely on a beetle emergence study (Huckaba and Storer 2008). However, BPPD noted other submitted studies conducted in greenhouse and field settings suggested that actual toxin doses may be lower than those assumed in the models (neither toxin has been considered to be a "high dose" toxin for CRW). BPPD is concerned that dose appeared to be a sensitive parameter in the models and simulations run with lower doses developed resistance more quickly than those with higher dose assumptions.
- Comparative modeling of different refuge sizes was not conducted. Most of the simulations assumed a 5% refuge for SmartStax. As such it was not possible to assess the value (or risk) of 5% refuge relative to 20% refuge (or other sizes).
- Recent selection experiments (Meihls et al. 2008) have suggested that resistance could evolve quickly with non-recessive inheritance. In light of these findings, BPPD suggested including higher initial resistance allele frequencies. For example, the stochastic model (Storer 2008) could include resistance allele frequencies > 0.001.

These uncertainties were conveyed to Monsanto/Dow in a letter from EPA dated March 19, 2009. Monsanto/Dow's response to these questions was received in a letter and attachment dated April 9. 2009. The review of that response follows below.

Monsanto/Dow Response to CRW IRM Concerns for SmartStax Corn

Monsanto/Dow's response to BPPD's review of SmartStax (BPPD 2009) is contained in a document titled Response to U.S. EPA BPPD letter, dated March 19, 2009 regarding applications to register MON 89034 x TC1507 x MON 88017 x DAS 59122-7..." (dated April 9, 2009). Additional clarifying information from Monsanto/Dow was submitted via e-mail (Russ Schneider to Alan Reynolds, dated 6/15/09). The response documents include information and data to address the following issues: 1) dose estimates (i.e. justification for the dose levels assumed in simulation models), 2) initial resistance allele frequency, and 3) simulation modeling and analysis of different refuge sizes for SmartStax (between 5 and 20%). BPPD's review of the proposal will concentrate individually on each of these areas.

1. Dose Estimates for SmartStax

Monsanto/Dow's estimates for dose mortality were derived from a field study submitted with the original SmartStax IRM proposal (Huckaba and Storer 2008). This study employed the dose calculation methods developed by Storer et al. (2006) in which dose is estimated by tabulating adult emergence in Bt and non-Bt plots (from a known artificial egg infestation) and correcting for density dependent mortality in the control plants. Based on this methodology, the authors concluded that the dose expression exceeded 99% (the lowest estimated dose was 99.238%) for Cry3Bb1 and Cry34/35 in both the single trait PIPs and SmartStax.

In its review of Monsanto/Dow's submission, BPPD noted that two other efficacy studies were submitted that also tabulated adult emergence from MON 89034, DAS 59122-7, and SmartStax. These studies (Vaughn et al. 2008; Clark and Harrison 2008) were not conducted with the methodology of Storer et al. (2006) and were not explicitly designed to estimate dose mortality. However, BPPD noted that adult emergence in some of the Bt plots relative to the non-Bt plots was higher than expected and might not be indicative of true 99% dose assumptions for Cry3Bb1 and Cry34/35, particularly in the single gene PIPs. To address BPPD's concerns over CRW dose assumptions, Monsanto/Dow was advised to provide either additional information to explain why the current dose (>99%) assumptions were justified, or to conduct additional model simulations using lower dose estimates. BPPD recommended dose levels of 85-95% for the single trait PIPs and 90-97% for the pyramid (SmartStax) for revised model simulations.

Monsanto/Dow has responded to these questions in the resubmission by providing a more comprehensive discussion of dose for CRW including how the observed data fit the dose assumptions used in the models. An explanation of the results of the other studies cited by BPPD (Vaughn et al. 2008; Clark and Harrison 2008) and their relevance to dose was also included. Finally, Monsanto/Dow submitted the results from a new model simulation incorporating revised dose parameters.

To help interpret Monsanto/Dow's dose estimates, the registrants provided a detailed discussion of the methodology used to calculate dose. As with the definition of dose used for lepidopteran pests, dose for CRW is described as the pest mortality caused by exposure to a PIP in the field. However, for CRW dose is complicated by the biology of the insect, particularly density-dependent and density-independent mortality caused by factors other than exposure to Bt toxins. Because of this, it is not possible to estimate dose by simply artificially infesting Bt and non-Bt corn plots and tabulating the percentage of larvae that emerge as adults in each treatment.

Density-dependent mortality impacts CRW in Bt and non-Bt fields differently. In Bt fields, larval populations (early instar) are reduced primarily by exposure to the Bt toxin. On the other hand, in non-Bt fields neonate larvae are not exposed to Bt, but population numbers are later reduced through high density-dependent mortality affecting primarily older instar larvae. The level of that mortality can exceed 95% (Onstad et al. 2006). CRW populations in Bt fields that have been reduced by mortality to Bt are not likely to

experience significant density-dependent mortality. To illustrate, the following diagram shows the likely causes of mortality for a hypothetical artificial infestation of 500 eggs in Bt and non-Bt plots (recreated from Figure 1 in Monsanto/Dow's submission, April 9, 2009):

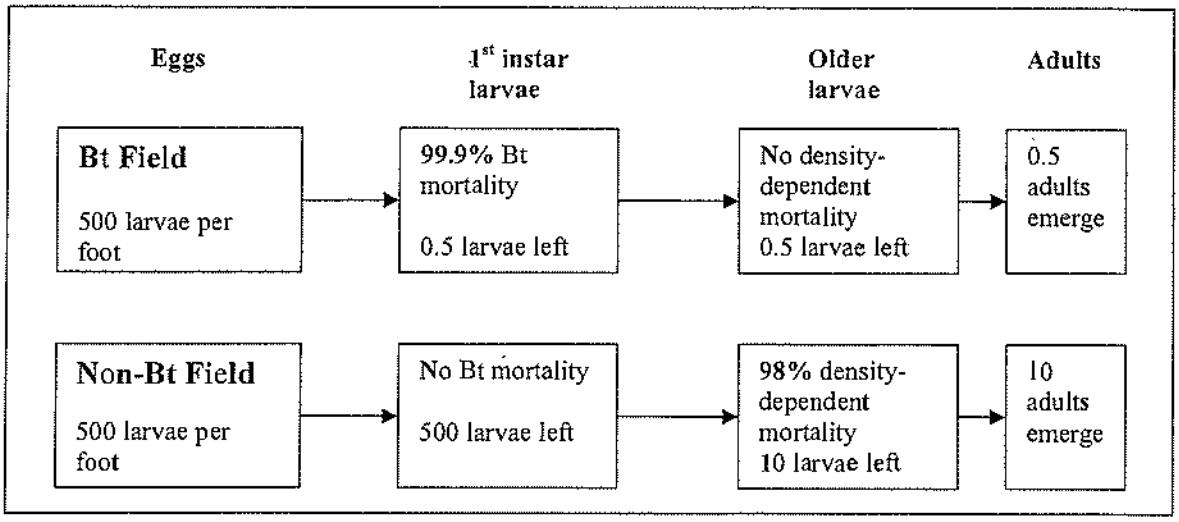

Figure 1. A schematic diagram following a cohort of 500 CRW larvae in an experiment to estimate dose. Reproduced from Figure 1 in Monsanto/Dow's submission.

Without correcting for density-dependent mortality, Monsanto/Dow assert that dose calculations would underestimate the true dose because adult emergence in non-Bt fields is suppressed by density-dependent mortality. The numbers of beetles that would emerge in non-Bt fields would be much higher if density-dependent mortality was not significant. In the example above, comparing only the adult emergence between Bt and non-Bt plots would produce a dose estimate of 95% (0.5/10). Monsanto/Dow argue that the correct comparison should occur for the first instar (i.e. before density-dependent mortality), which would result in a dose estimate of 99.9% (0.5/500).

Density-dependent mortality has been assessed by tabulating the proportion of eggs that emerge as adults from artificially-infested Bt plots. Much of the work on the relationship between egg density and adult emergence was conducted by Onstad et al. (2006) who showed that increasing egg density lowers the percentage of emerging adults. Regression curves for the egg density/adult relationship indicate that density-dependent mortality becomes significant when egg densities exceed several hundred eggs per row foot of corn (refer to Figure 2 in Monsanto/Dow's submission).

In the review of the original SmartStax IRM submission, BPPD noted that although multiple greenhouse or field efficacy trials were submitted (in which adult emergence was tabulated) only one study (Huckaba and Storer 2008) was used by Monsanto/Dow to determine dose. The other studies (Clark and Harrison 2008; Vaughn et al. 2008) appeared to show adult emergence that was inconsistent with dose estimates derived from Huckaba and Storer (2008) when CRW emergence in Bt treatments was compared to

non-Bt treatments. For some plots in the Vaughn et al. (2008) study, the reduction in emergence relative to the control was less than 90% (as low as 72.1% in one case).

Monsanto/Dow emphasize that both of these studies were designed for other purposes and cannot reliably be used to determine dose mortality levels. The root mat experiment (Clark and Harrison 2008) was conducted in greenhouses under optimal growth conditions for corn plants and CRW to determine additive or non-additive activity of the toxins in the SmartStax pyramid. Due to the favorable growth climate, the companies believe that the plant root systems developed abundant root sites with low Bt expression (presumably secondary roots or root hairs) that allowed for full development of CRW larvae. Density-independent mortality was also minimized during the experiment with the prime soil and plant conditions. Monsanto/Dow suggest that CRW survival in this test system could be an order of magnitude greater than what would be expected in a true field environment. Further, density-dependent mortality would follow different patterns in the greenhouse compared to field conditions.

The Vaughn et al. (2008) study was a field trial; however, in contrast to Huckaba and Storer (2008) the study was conducted using natural CRW infestation rather than controlled artificial infestations. Testing locations were selected based on predicted high levels of pest infestation. Since these naturally-occurring CRW populations were expected to be highly variable, it was unfeasible to accurately determine the infestation level prior to exposure to the Bt treatments. Likewise, it was impossible to estimate the level of density-dependent mortality in the non-Bt control plots. Without this information, a reliable calculation of dose mortality cannot be made. As for the adult emergence patterns that were observed in the study, Monsanto/Dow believe the results were still consistent with their expected dose levels for the Bt corn events. The registrants used Storer's stochastic model (Storer 2008; initially reviewed in BPPD 2009) to estimate the expected reduction in adult emergence and number of adults (per plant) expected to emerge at differing dose levels (the graphical curves are contained in Figures 3 and 4 of Monsanto/Dow's submission). Assuming that infestation levels were high in the tests (5x normal infestation density), the worst case percent emergence reduction (~ 75%) observed in Vaughn et al. (2008) would correspond to a ~ 98% dose. (At "normal" infestation levels, 75% emergence reduction would equate to a ~ 90% dose.) In terms of number of adults emerging per plant, Monsanto/Dow note that in most of their trials, Bt plots produced on average less than one adult per plant. This emergence corresponds to dose levels >99% at both low and high infestation levels. Even a dose of 99% under heavy infestation could result in up to 3 adults emerging per plant.

In contrast to the Clark and Harrison (2008) and Vaughn et al. (2008) studies, Huckaba and Storer (2008) was designed specifically to determine dose mortality. Egg infestation was controlled and tabulated so that density-dependent mortality could be included in the calculations. Field tents were used to trap and count emerging adults in each plot. Monsanto/Dow contend that the results of Huckaba and Storer (2008) for DAS-59122 (Cry34/35) were consistent with those obtained in Storer et al. (2006) using the same methodology. Furthermore, a recent study (Meihls et al. 2008) reported that survival on

Cry3Bb1 corn plants was 4.2% (and 1.34% relative to non-Bt corn), similar to the observations in Huckaba and Storer (2008).

Repellency and/or other non-lethal modes of action have been suggested to describe the efficacy of CRW Bt toxins. However, Monsanto/Dow argue that mode of action should be considered separately from dose because ultimately Bt (through repellency or other chronic effects) is still the cause of mortality (based on the conclusions of Whalon and Wingerd 2003).

BPPD Review - Dose Estimates

The new information on dose presented by Monsanto/Dow in their resubmission is helpful for clarifying the issue of dose expression in SmartStax (and its single trait components). This information also provides further support for the dose estimates used in Monsanto/Dow's simulation models for SmartStax. However, BPPD believes that there is still some uncertainty in the dose estimations for CRW, largely due to the difficult nature of assessing larval response and behavior in a subterranean environment.

BPPD agrees with Monsanto/Dow that the methodology used in Storer et al. (2006) and Hucakaba and Storer (2008) is a reasonable approach to address dose for CRW (i.e. artificial egg infestation in Bt and non-Bt plots, emergence tents to capture/count adult emergence, and adjustments for density-dependent mortality in non-Bt plots). Further, BPPD agrees that the other reviewed efficacy studies (Clark and Harrison 2008; Vaughn et al. 2008) were not designed to assess dose and cannot by themselves be used to quantitatively determine dose expression. Still, using a weight-of-evidence approach, BPPD believes these studies should be considered in the discussion of dose mortality for SmartStax.

Regarding the Vaughn et al. (2008) study, BPPD understands that the test was conducted with natural infestation (and not controlled artificial infestation) so that a quantitative estimate of dose level could not be determined. Also, the results fit within the expected adult emergence pattern based on Storer's stochastic model (Storer 2008), which can explain much of the observed adult emergence in Bt plots. Using this model, the "worst case" test plot (in which the reduction in emergence in a Bt plot relative to the control was 72.1%) would still correspond to a dose level of approximately 98% (under "high density" infestation). On the other hand, BPPD notes that under "normal" infestation levels, the expected dose would fall to approximately 91% (refer to Figure 3 in Monsanto/Dow's submission). It is unclear from the data or the report whether this site experienced normal or high CRW infestation. Since this site had the highest adult emergence in a Bt plot (relative to the control), it could be representative of the lowest boundary for a dose estimate for a worst case scenario (i.e. 91 to 98% depending on infestation levels). BPPD emphasizes that the single site with 72.1% reductions was an outlier -- most of the other tested sites in Vaughn et al. (2008) had emergence levels that would correspond to dose estimates exceeding 98%.

The root mat study (Clark and Harrison 2008) is more difficult to interpret. Again, BPPD agrees with Monsanto/Dow that the study was not specifically designed to assess dose and was conducted under greenhouse conditions optimized for corn and CRW survival. Monsanto/Dow's suggestion that the root mat created ample "feeding sites with low Bt exposure" is plausible, though no data were presented to verify this assumption. Density-independent mortality should be greatly reduced in such settings (as indicated by Monsanto/Dow) but should be considered separately from dose (i.e. both CRW on Bt and non-Bt root mats should be affected equally). BPPD agrees that overall CRW survival should be higher in the greenhouse than in the field but is still concerned about the relative differences in emergence between treatments. Density-independent mortality (or lack thereof) and optimized growth conditions should impact CRW in Bt and non-Bt treatments equally. Nevertheless, the adult emergence in both the single and root mat treatments did not appear to equate with dose estimates of 98% or higher (refer to Table 3 in BPPD 2009).

Given the difficult task of quantitatively determining dose for CRW, the methodology of Storer et al. (2006) (as employed in Huckaba and Storer 2008) may be the best means presently available to estimate dose mortality. A key aspect of this technique is to adjust for density-dependent mortality in non-Bt treatments (exposure to Bt is assumed to reduce CRW populations below the level of significant density-dependent mortality. However, there is some disagreement about the egg density level that triggers density-dependent mortality. Monsanto/Dow (and Storer et al. 2006) have cited the density-dependent morality curves developed by Onstad et al. (2006) in which density-dependent mortality starts to dramatically increase at 200-300 eggs/foot (refer to Figure 2 in Monsanto/Dow's submission). With the density-dependent adjustment, the dose estimates for DAS 59122-7, MON 88017, and SmartStax exceed 99% in both Storer et al. 2006 (Cry34/35 only) and Huckaba and Storer 2008 (Cry3Bb1 and Cry34/35). On the other hand, data developed by Dr. Bruce Hibbard (personal communication, unpublished data) suggest that density-dependent mortality may not occur until egg density exceeds 850 eggs/foot. Monsanto/Dow's dose trials (Huckaba and Storer 2008) used an infestation rate of 1006.5 eggs per row foot. If a 75% egg viability rate is assumed (as the researchers did) the infestation rate would fall to 750 eggs/foot, which would be below the density-dependent mortality threshold suggested by Hibbard. In addition, BPPD notes that density-independent mortality (separate of dose effects) can be expected to also reduce CRW abundance in both Bt and non-Bt plots, perhaps further decreasing the likelihood of density-dependent mortality. Without density-dependent mortality included, Huckaba and Storer (2008) would yield dose estimates of 96.17 - 99.96% (MON 88017), 94.20 - 99.18% (DAS 59122), and 98.22 - 99.97% (SmartStax pyramid).

Based on the discussion above, BPPD concludes that the low end of the dose range estimates of 96.2 (MON 88017/Cry3Bb1), 94.2% (DAS 59122/Cry34/35), and 98.2 (SmartStax/Cry3Bb1 & Cry34/35) are reasonable for modeling. These estimates are lower than those originally advocated by Monsanto/Dow but are largely within the ranges tested in the revised model included in the resubmission (see discussion later in this review). Although the dose estimates used in the revised modeling may be supported based on the empirical data in Huckaba and Storer (2008), BPPD believes that

Monsanto/Dow may still not have conducted a truly "worst case" model scenario. As previously discussed, it is still not entirely clear how the results observed in the root mat and natural infestation field test comport with the dose estimates assumed in the model. Also, BPPD notes that dose has been described only for western corn rootworm; efficacy against northern corn rootworm has been shown to be lower in some cases with Cry34/35 corn (see BPPD 2005b). A worst case modeling scenario (i.e. using dose levels of 91% for the single traits) could also address any uncertainty with northern corn rootworm.

Despite some uncertainty, BPPD notes that there is currently no evidence (from empirical data) that the dose levels are below 94% for the single traits (in BPPD 2009, it had been previously suggested that using a single trait dose level of 85% might be appropriate). Rather, the data developed to date suggest that the dose ranges (without density-dependent mortality corrections) described above could be realistic for Cry34/35 and Cry3Bb1. For the SmartStax pyramid, BPPD concludes that a dose level of 98.2% is realistic based upon the available evidence (BPPD had previously suggested using a dose level of 90% for the pyramid).

Despite Monsanto/Dow's arguments, BPPD believes that the mode of action of Bt toxins against CRW could influence the issue of dose. Non-lethal means, such as repellency or other behavioral responses, or chronic effects could impact the way in which CRW adapts (or becomes resistant) to Bt corn. Of particular interest could be the development of cross resistance to multiple toxins due to behavioral modifications. However, given the lack of data in this area, the effects of mode of action remain an uncertainty in the discussion of dose.

Overall, BPPD concludes that there is still uncertainty regarding dose determinations for CRW. Storer et al. (2006) is currently the best approach to evaluating dose, but limited data have been developed using this approach. BPPD notes that two studies have been conducted for Cry34/35 (Storer et al. 2006 and Huckaba and Storer 2008), but only one year (with six locations) of data were developed for Cry3Bb1 (Huckaba and Storer 2008). Other *Diabrotica* spp. may also need to be investigated: data submitted for northern corn rootworm revealed mortality as low as 92.8% on Cry34/35 (BPPD 2005b). To address these uncertainties and buttress the dose assumptions used in the models, BPPD recommends that Monsanto/Dow conduct additional studies to investigate dose for CRW, particularly for Cry3Bb1. Further dose studies could also be conducted with varying egg infestation levels (above and below egg levels expected to trigger density-dependent mortality) to tease out any egg density effects. New techniques to assess CRW dose may need to be pursued as well, if Monsanto/Dow or academic researchers can develop such approaches.

2. Initial Resistance Allele Frequency

BPPD's review of Monsanto/Dow's initial SmartStax IRM proposal raised concerns that the initial resistance allele frequency assumed by the simulation modeling might be too low based on recent selection experiments (BPPD 2009). Monsanto/Dow maintain that

an initial resistance allele frequency of 0.001 is realistic, as was assumed in the models submitted to in the original SmartStax IRM submission (see Storer 2008). They note that a 0.001 resistance allele frequency has been assumed as a conservative value in IRM modeling for other pests and Bt crops. Further, selection experiments conducted in greenhouses (under optimal CRW growth conditions) could select for different resistance alleles than might occur in the field.

To address BPPD's concern, Monsanto/Dow conducted model simulations with Storer's stochastic model (see Storer 2008) with varying initial resistance allele frequencies (0.01, 0.005, and 0.001). Simulations were run with the single traits (dose assumption 99.75% each) and the SmartStax pyramid (dose assumption 99.95%) to determine the resistance allele frequency (RAF) after 10 generations. A 5% refuge for the pyramid and 20% refuge for the single traits were also assumed. The results showed that as expected, with the single traits the final RAF after 10 generations of selection was higher when the initial frequency was higher. For example, when the initial RAF was 0.001, the final RAF after 10 generations was slightly below 0.01. But an increase of the initial RAF to 0.01 resulted in a final RAF of close to 1.0 (i.e. total resistance) after 10 generations. Unlike the single trait simulations, the results for modeling with the pyramid showed that the final RAF did not increase significantly from the initial frequency after 10 generations of selection. This was true for all of the initial RAF values (0.01, 0.005, and 0.001). To illustrate, the model runs with a 0.01 initial RAF resulted in a final RAF of approximately 0.01 (i.e. almost no increase in frequency). A summary of the model output from these simulations is contained in Figure 7 of Monsanto/Dow's submission.

Based on the model results, Monsanto/Dow conclude that initial resistance allele frequency is an insensitive parameter in the model for the pyramid (relative to the single toxin traits). This is largely because multiple resistance alleles are needed to overcome the pyramid -- individuals must be homozygous resistant for one allele and heterozygous for the second (or homozygous resistant for both alleles). The frequency of homozygote individuals for both resistance alleles should be extremely rare (1×10^{-8} for a 0.01 initial RAF assumption). Even insects homozygous resistant for one allele and heterozygous for the other are expected to occur at frequency of only 1×10^{-6} when the initial RAF is 0.01.

BPPD Review - Initial Resistance Allele Frequency

Although Monsanto/Dow present a logical rationale for their assumptions regarding resistance allele frequencies, BPPD is still concerned that resistance alleles for CRW-targeted Bt traits may be relatively common in the field. Published CRW selection studies (Lefko et al. 2008; Meihls et al. 2008) suggest that resistance alleles to Cry34/35 and Cry3Bb1 occur relatively frequently, as populations resistant or tolerant to the toxins were selected within 10 generations. While a resistance allele frequency assumption of 0.001 may be suitable for other pests (e.g. lepidoptera), BPPD must consider the possibility that actual RAF for CRW is higher (perhaps close to 0.01).

Monsanto/Dow's modeling clearly demonstrates that the initial RAF has more importance for single trait PIPs than a pyramid (SmartStax). Since two or more resistance genes would be needed to overcome a pyramid, resistant individuals should be rare even with a higher (0.01) RAF for both individual toxins. However, should SmartStax be registered, it will be deployed in a landscape that will also contain significant acreage of single trait PIPs (including DAS 59122 and MON 88017). If resistance alleles for Cry34/35 and/or Cry3Bb1 are common, CRW may be able to develop resistance to one of the single trait PIPs, which could then serve as a "stepping stone" for resistance to the pyramid. BPPD also notes that the model simulations to investigate initial RAF were conducted with the high dose assumptions (99.75% for single traits and 99.95% for the pyramid). It is unclear how the model output would be affected with lower dose assumptions, such as those described previously in this review.

Other recently conducted modeling scenarios with pyramided traits (Onstad 2009 - draft) suggest that the benefits of pyramids (i.e. delay in resistance development) may be lower when the initial RAFs for the individual toxins are high (close to 0.1). In these cases, the time to resistance was reduced for both low and high (99.9%) dose assumptions (high dose traits actually evolved resistance slightly more quickly than medium or lose dose traits). Conversely, pyramids provide better resistance management in a landscape with single trait PIPs when the initial RAF is low (0.0001). While BPPD believes an initial resistance allele frequency of 0.1 is extremely unlikely for resistance to Cry34/35 or Cry3Bb1, this modeling demonstrates the potential effects higher RAFs can have on the development of resistance.

BPPD agrees with Monsanto/Dow that laboratory (greenhouse) selection experiments may yield a different type of resistance than might evolve under field conditions. But in this case, BPPD is not aware of any empirical data for Cry34/35 or Cry3Bb1 from field-level selection that would indicate resistance alleles are rare (i.e. ≤ 0.001). BPPD recommends that Monsanto/Dow develop and conduct CRW selection studies to investigate potential resistance. Such data could help determine realistic resistance allele frequencies for Cry34/35 and Cry3Bb1 (and other assumptions about resistance) to better refine simulation models.

3. Resistance Modeling

BPPD's assessment of Monsanto/Dow's initial modeling (Storer 2008) for SmartStax corn expressed concerns that unrealistic assumptions may have been included regarding dose and initial resistance allele frequency (see BPPD 2009 and discussions in the previous two sections). In response to BPPD's conclusions, Monsanto/Dow conducted an additional model simulation using lower dose estimates.

The new simulations utilize the same stochastic model structure (Storer 2008) that was previously developed for SmartStax. Monsanto/Dow used dose estimates based on the data obtained from Huckaba and Storer (2008) (as previously modeled) but without the adjustments for density-dependent mortality. Further, the registrants selected the location

(York, NE) that produced the lowest overall dose estimates in the Huckaba and Storer (2008) study. This adjustment produced dose values of 97.5% for Cry3Bb1, 94.2% for Cry34/35, and 98.2% for the Cry3Bb1/Cry34/35 pyramid. By reducing the dose levels, the functional dominance of Cry3Bb1 increased to 0.269 and Cry34/35 increased to 0.396. Monsanto/Dow indicated that these dominance levels are comparable to those calculated for Cry3Bb1 in the selection experiments of Meihls et al. (2008). Initial resistance allele frequency was held at 0.001 for each of the toxins (the same as in the previous modeling).

Model output from the revised simulation was similar to the results observed in the initial modeling. The single trait PIPs (in a mosaic with a 20% refuge) developed resistance (defined as RAF $\geq$ 0.1) within 10 years. On the other hand, the pyramid (with a 5% refuge) drove the populations to extinction before resistance could develop to either toxin. The results are shown in Figure 2 below (reprinted from Monsanto/Dow's submission).

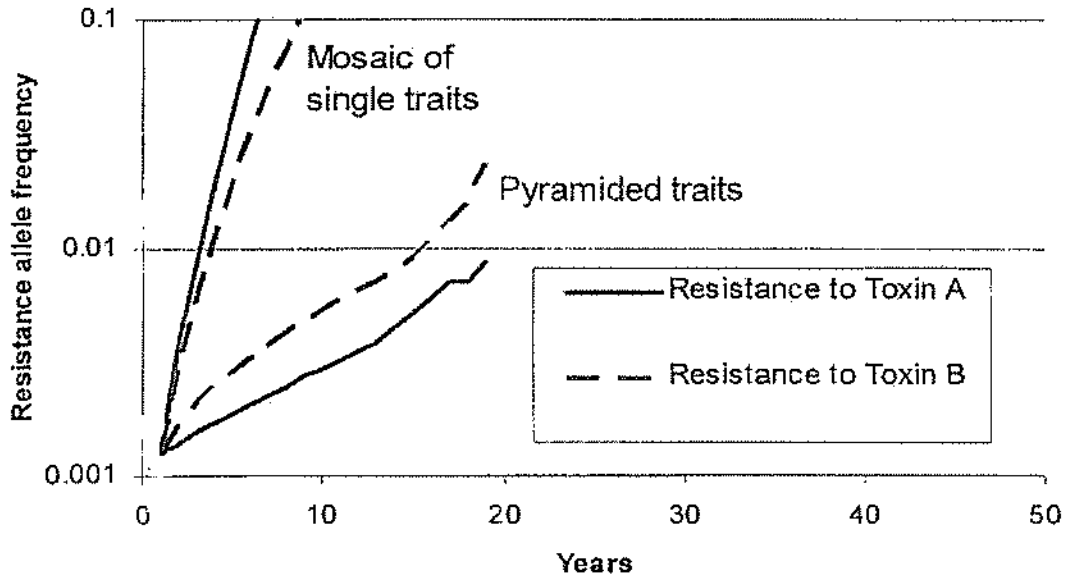

Figure 2. Results from Monsanto/Dow's model with revised dose estimates (97.5% for Cry3Bb1, 94.2% for Cry34/35, and 98.2% for the Cry3Bb1/Cry34/35 pyramid). Reprinted from Figure 5 in Monsanto/Dow's submission (April 9, 2009).

In addition to the simulation with revised dose estimates, Monsanto/Dow also addressed BPPD's request to conduct modeling to compare various refuge sizes. Two dose scenarios were investigated including high (0.997) and low (0.968) estimates for the single trait PIPs. The refuge sizes were 5, 10, and 20% for the pyramid and 20% for the single traits. Monsanto/Dow also included a range of values for potential functional dominance of the resistance trait (0.06 - 0.32). Under the high dose assumption (0.997), there was essentially no difference in the time to resistance (i.e. resistance did not evolve within 100 generations) regardless of the refuge size or functional dominance. Single trait PIPs, on the other hand, developed resistance relatively quickly (<20 generations) for

all levels of functional dominance. With the lower dose estimate (0.968), functional dominance had more of an effect on the output. At higher levels of functional dominance (0.18 and 0.32), smaller refuge sizes developed resistance more quickly, though a pyramid with a 20% refuge remained robust (i.e. no resistance within 100 generations). Regardless, in all scenarios with the lowest refuge for a pyramid (5%), resistance was still significantly delayed compared to the single traits with a 20% refuge. The model results are shown in Figure 3 below (reprinted from Monsanto/Dow's submission).

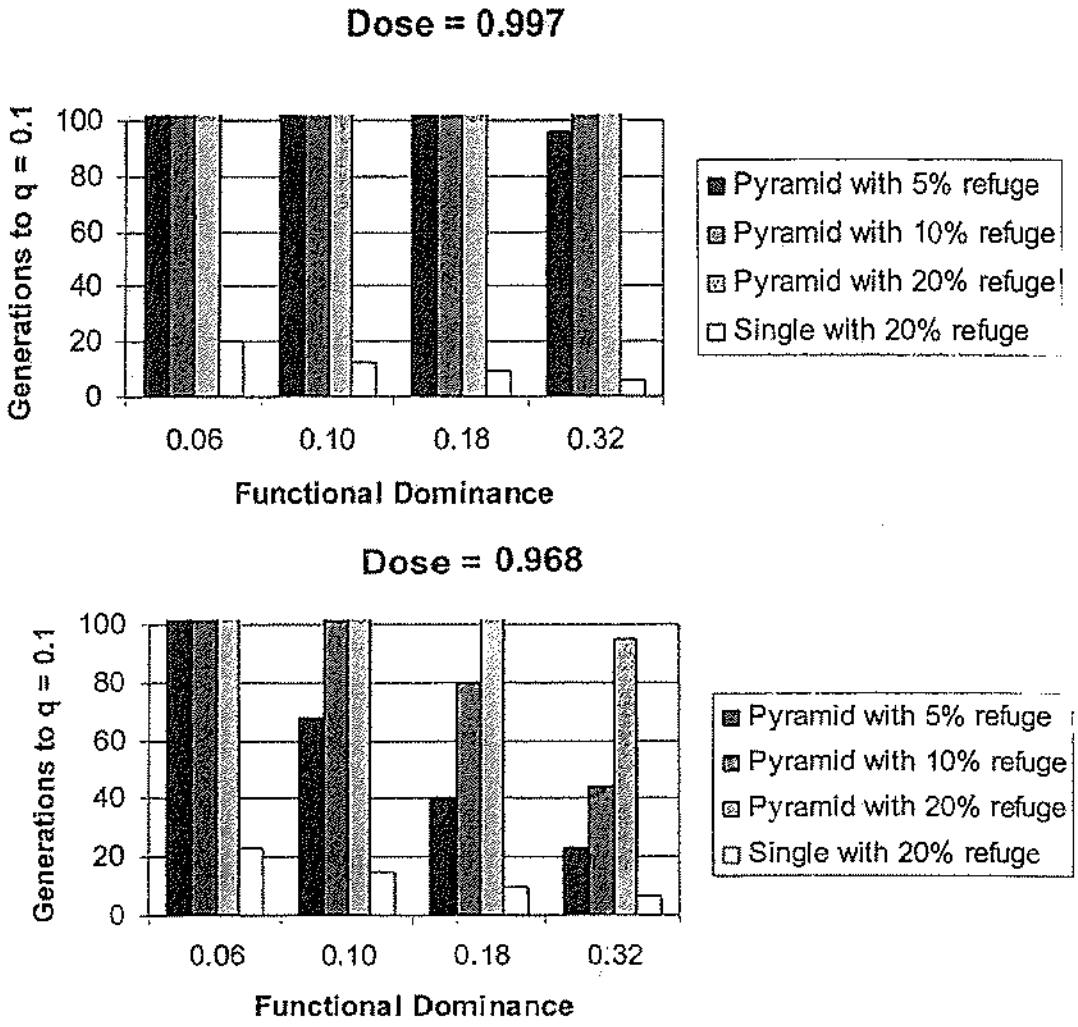

Figure 3. Results from modeling with various refuge sizes (5, 10, and 20%) under two dose scenarios (0.997 and 0.968). Reprinted from Figure 5 in Monsanto/Dow's submission (April 9, 2009).

BPPD Review - Revised Modeling

BPPD agrees with Monsanto/Dow's use of lower dose estimates based on data without adjustments for density-dependent mortality (see the previous discussion in the dose section). Without density-dependent mortality included, the dose data (Huckaba and

Storer 2008) would yield dose estimates of 96.17 - 99.96% (Cry3Bb1), 94.20 - 99.18% (Cry34/35), and 98.22 - 99.97% (SmartStax pyramid). Monsanto/Dow's revised simulation included dose values within these ranges for each of the toxins. With these new dose estimates, the model output was largely the same as the previous simulations and showed that a pyramid with the two toxins and a 5% refuge was less likely to develop resistance than a mosaic of single traits with 20% refuges.

Independent of dose, the initial resistance allele frequency (0.001) that was used in the original model was maintained for the new simulations. BPPD had recommended using a more conservative value of 0.01 (as discussed in the preceding section). It remains unclear what effect using 0.01 as the initial RAF would have on the model output. BPPD assumes that the time to resistance would decrease for both the single trait mosaic and the pyramid deployment scenarios, though the pyramid would remain more durable than the single traits. It is also possible that the populations exposed to the pyramid might not go to extinction as they did with a 0.01 initial RAF. Regardless, it would be useful to determine the relative differences between the single trait mosaic and pyramid simulations with an assumption of a higher initial RAF.

IRM models (including those developed by Monsanto/Dow for SmartStax and other researchers) developed to date support the concept that pyramided toxins provide superior resistance management compared to single trait PIPs. These models (Roush 1998, Zhao et al. 2003, Gould 2006, and Storer 2008) show that pyramids of two traits with independent modes of action (i.e. no cross resistance potential) and high activity (dose) delay the evolution of resistance relative to single traits. The first of these criteria (no cross resistance) has been addressed for SmartStax (see discussion in BPPD 2009). For the second criterion (dose), BPPD concludes that Monsanto/Dow's more conservative dose estimates are reasonable but recognizes that there is still uncertainty over the issue for SmartStax.

Monsanto/Dow's comparative modeling for refuge sizes was illuminating. Should the higher dose estimates hold for the SmartStax toxins (i.e. >99%), there appears to be little difference (or value added) between refuge sizes for a pyramid. However, the lower dose estimate (0.968) could be a more likely value based on the dose data reviewed to date (see previous dose discussion). In this case, there were differences between refuge sizes, with larger refuges for the pyramid (10 and 20%) providing more durability than the 5% refuge (see Figure 3 above). Still, it is important to note that the time to resistance of the 5% refuge was still much greater than that for the single trait PIPs with a 20% refuge. In the worst case scenario (functional dominance = 0.32), the time to resistance for the 5% pyramid refuge is approximately 22 generations while the 20% refuge for single traits evolved resistance within about 7 generations. This modeling (like many of the other simulations) clearly demonstrates the improved durability of pyramids relative to single traits regardless of dose or refuge assumptions.

Another recent modeling effort has been completed by Onstad (2009, draft) to evaluate the effectiveness of pyramids for corn rootworm resistance management. This model simulated a variety of dose estimates, refuge sizes, initial resistance allele frequencies,

and deployment scenarios. Largely, the results of Onstad's model corroborated those from other models in that pyramids were shown to delay resistance longer than single traits in most cases. Interestingly, refuge size (5, 10, and 20% refuges were modeled) had only a limited effect on time to resistance, though in most scenarios larger refuges delayed resistance somewhat longer than smaller (5%) sizes. As with previous models, dose appears to have been a sensitive parameter. A range of doses were simulated from higher levels (99.9% mortality) to lower estimates (80 - 95% mortality). In general, higher doses delayed resistance longer than lower doses in most simulations, though in some cases the lowest dose (80%) delayed resistance longer than the intermediate dose (95%). The initial resistance allele frequency also had a major effect on the output -- in scenarios with pyramided and single trait fields, a higher initial RAF (0.1 in the model) led to much quicker resistance (60-70% faster) than a lower initial RAF (0.0001). Onstad concluded that a high initial RAF may erode the benefits of a pyramid relative to the sequential introduction of single trait PIPs. Finally, the time to resistance was greatly reduced when insecticides were used in refuges -- a common practice with structured corn rootworm refuges.

Overall BPPD Conclusions for SmartStax Corn Rootworm Refuge

Considering all of the data and information submitted to support the proposed corn rootworm refuge (reviewed here and in BPPD 2009), BPPD concludes that there is sufficient scientific justification to support a reduced refuge (to 5%) for SmartStax corn. Though larger refuge sizes for pyramids (e.g. 10%) may delay resistance longer, the weight-of-evidence clearly indicates that a 5% pyramid refuge is superior to the currently mandated refuges of 20% for single trait PIPs. However, because of uncertainty with some aspects of the proposal (as described below and in this review), BPPD recommends that additional data be developed to verify and further support the current data set for reduced refuge.

IRM models (Roush 1998, Zhao et al. 2003, Gould et al. 2006, Onstad 2009 - draft) have consistently shown that pyramids (with two or more toxins) provide superior durability compared with single trait PIPs, provided that the toxins have 1) no cross resistance and 2) are individually expressed at high enough levels in the pyramid. Previously submitted data (reviewed in BPPD 2009) have demonstrated that Cry34/35 and Cry3Bb1 have little or no cross resistance potential, which fulfills the first assumption for an effective pyramid. The second criterion, dose, has been more difficult to evaluate. CRW biology is not conducive to simple laboratory or field experiments to assess dose since larvae develop in a subterranean environment. Larval infestations in the field are often great enough to trigger density-dependent mortality. Further, the mode of action of CRW-active toxins is not entirely understood and it is unclear whether repellency or other behavioral responses may influence the assessment of dose. As such, the methodology used to determine dose for lepidopteran corn pests is not suitable for CRW and alternate approaches have been considered.

BPPD agrees with Monsanto/Dow that the technique developed by Storer et al. (2006) to assess CRW dose is reasonable. A key component of this method is an adjustment for density-dependent mortality in non-Bt plots above a certain egg infestation level, though there is conflicting information on the exact threshold that triggers density-dependent mortality (see Onstad et al. 2006 and Hibbard, personal communication). As discussed in the dose section, the unadjusted data from Monsanto/Dow's dose study provide a more conservative measure of dose and result in estimates of 96.17 - 99.96% (Cry3Bb1), 94.20 - 99.18% (Cry34/35), and 98.22 - 99.97% (Cry3Bb1 and Cry34/35 pyramid). If these dose levels are considered in the modeling, the second key assumption for an effective pyramid will have been fulfilled.

Although BPPD concludes that the dose values above are good estimates for the SmartStax toxins, there is still some uncertainty regarding dose. Limited data have been developed using the Storer et al. (2006) methodology. BPPD notes that two studies have been conducted for Cry34/35 (Storer et al. 2006 and Huckaba and Storer 2008), but only one year (with six locations) of data were developed for Cry3Bb1 (Huckaba and Storer 2008). Other *Diabrotica* spp. may also need to be investigated: data submitted for northern corn rootworm revealed mortality as low as 92.8% on Cry34/35 (BPPD 2005b). To address the uncertainty with dose, BPPD recommends that Monsanto/Dow conduct additional studies to investigate dose for CRW, particularly for Cry3B1.

Monsanto/Dow have addressed another of BPPD's major concerns by conducting revised model runs (based on Storer 2008) incorporating the lower dose estimates described above (i.e. uncorrected for density-dependent mortality) with little change to model output (resistance was not observed for SmartStax with a 5% refuge). Other modeling previously developed by Monsanto/Dow (Gustafson and Head 2008) has also supported the durability of the SmartStax pyramid under certain dose assumptions. Comparative modeling with different refuge sizes suggests that under some circumstances, higher refuges (10 and 20%) for pyramids may delay resistance longer than a 5% refuge. However, the 5% refuge delayed resistance much longer than a 20% refuge with single trait PIPs in all of the modeled scenarios. Overall, these model runs (and other published models) provide strong evidence that pyramided PIPs are superior to the current single trait products and can justify a lower CRW refuge for the SmartStax pyramid.

Despite Monsanto/Dow's these conservative simulations, BPPD remains concerned that "worst case" scenarios for SmartStax have not yet been fully investigated in the modeling. CRW-protected corn is highly adopted in some areas with heavy infestations so that intense selection pressure for resistance can be expected. In light of this and the large proposed reduction in refuge (from 20% to 5%; a 75% total reduction), BPPD believes that worst case analyses are warranted to help determine the potential for resistance. In particular, model parameters for dose and initial resistance allele frequency could be adjusted to include more conservative estimates. Scenarios with lower assumed doses could account for less sensitive pests (i.e. northern corn rootworm) or add a margin of error should subsequent data reveal previous dose assumptions to be overestimates. Also, the dose level that would cause the risk of resistance with a pyramid to significantly increase is unclear in the Storer (2008) model. The initial resistance allele frequency

(0.001) in Storer's model may be set too low -- published resistance selection studies (Lefko et al. 2008, Meihls et al. 2008) have suggested resistance alleles may be relatively common in field populations. BPPD notes that both dose and initial resistance allele frequency are sensitive parameters in Monsanto/Dow's model (Storer 2008) and other models -- modest changes to both can greatly affect the predicted durability in a typical model scenario.

To address the remaining uncertainty in the SmartStax IRM proposal, BPPD recommends: 1) additional dose studies (as discussed above); 2) selection experiments to further characterize putative resistance alleles and frequency of occurrence in CRW populations, and 3) revised (worst case) resistance modeling based on the concerns described above and any new information from dose and selection experiments or other available research. Further, to improve the overall risk assessment for SmartStax, BPPD strongly recommends that a reassessment of the available data (including any newly conducted studies) be conducted within several years of approval to confirm the suitability of a 5% refuge.

Should SmartStax be registered with a 5% refuge requirements for lepidoptera and CRW, the product will have different refuge requirements then other registered Bt corn products. Therefore, additional aspects of the IRM program for SmartStax including compliance and grower education will need to be developed due to accommodate the different refuge size. Also, the existing programs for resistance monitoring and remedial action that were established for MON 89034 (Cry1A.105 and Cry2Ab2), MON 88017 (Cry3Bb1), and Herculex Xtra (Cry1F and Cry34/35) should be applicable to SmartStax corn. However, BPPD recommends that this program be expanded (i.e. with additional sampling and collection sites or improved monitoring techniques). BPPD believes that resistance monitoring will have added importance with less overall structured refuge for Bt corn. Also, a revised definition of "resistance" may be needed for the CRW monitoring and remedial action plans based on recent research and selection experiments.

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